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(57) Abstract

A predetermined molecular structure in a sample is detected by sensing a pattern in which the sample binds to an array of binding sites in a molecular detection device (20), and comparing the pattern to a reference pattern to detect the predetermined molecular structure in the sample (22). In one embodiment, the reference pattern is generated by sensing a pattern in which a reference sample containing the predetermined molecular structure binds to a like array of binding sites. In another embodiment, the reference pattern is generated by predicting a pattern in which the predetermined molecular structure binds to the array of binding sites.

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METHOD AND APPARATUS FOR DETECTING PREDETERMINED MOLECULAR STRUCTURES IN A SAMPLE

Field of the Invention

The present invention relates to methods and system for molecular detection.

Background of the Invention

An increased effort has been directed toward the development of chips for molecular detection. Typically, a molecular detection chip includes a substrate on which an array of binding sites is arranged. Each binding site, or hybridization site, has a respective molecular receptor which binds or hybridizes with a molecule having a predetermined structure.

A sample solution is applied to the molecular detection chip, and molecules in the sample bind or hybridize at one or more of the binding sites. The particular binding sites at which hybridization occurs are detected, and one or more molecular structures within the sample are subsequently deduced.

Of great interest are molecular detection chips for gene sequencing. These chips, often referred to as DNA chips, utilize an array of selective binding sites each having respective single-stranded DNA probes. A sample of single-stranded DNA fragments, referred to as target DNA, is applied to the DNA chip. The DNA fragments attach to one or more of the DNA probes by a hybridization process. By detecting which DNA probes have a DNA fragment hybridized thereto, a

sequence of nucleotide bases within the DNA fragment can be determined.

One use of molecular detection chips is to perform medical diagnostics. Here, a genomic sample from an individual is screened to determine if the individual has a genetically-inherited disease or a genetic predisposition to disease.

As medical diagnostics migrate to miniature devices capable of performing higher numbers of tests with greater sensitivity, there is a corresponding increase in the amount of information being generated, and in the demand for greater processing power to obtain test results.

Brief Description of the Drawings

The invention is pointed out with particularity in the appended claims. However, other features of the invention will become more apparent and the invention will be best understood by referring to the following detailed description in conjunction with the accompanying drawings in which:

FIG. 1 is a flow chart of an embodiment of a method of detecting a predetermined molecular structure in a sample;

FIG. 2 is a flow chart of an embodiment of a method of generating the reference pattern;

FIG. 3 is a flow chart of another embodiment of a method of generating the reference pattern;

FIG. 4 illustrates an array of binding sites on a molecular detection device used in the specific embodiment;

FIG. 5 illustrates a reference pattern for detecting an a-c-g nucleotide sequence in a sample;

FIG. 6 illustrates a reference pattern for detecting an a-c-t nucleotide sequence in a sample;

FIG. 7 is an example of a pattern generated by a sample having an unknown molecular structure;

FIG. 8 is a flow chart of additional steps which can be utilized to detect the predetermined molecular structure;

FIG. 9 is a block diagram of an apparatus for detecting a predetermined molecular structure in a sample; and

FIG. 10 is a flow chart of an embodiment of a method of gene discovery in accordance with the present invention.

Detailed Description of a Preferred Embodiment

Embodiments of the present invention advantageously provide improved information processing approaches to detecting predetermined molecular structures using a miniaturized device having an array of biological sensors. Just as semiconductor devices are designed to perform specific functions, a diagnostic device in accordance with the present invention is designed to perform one or more specific diagnostic tests.

FIG. 1 is a flow chart of an embodiment of a method of detecting a predetermined molecular structure in a sample. In general, the method can be utilized for detection of a variety of molecular structures in a variety of different types of samples. Examples of the different types of samples include, but are not limited to, medical samples, environmental samples, agricultural samples, and other samples applicable to diagnostics.

The predetermined molecular structure can be any indication of a structure of molecules contained in the sample. For example, the predetermined molecular structure can be indicative of a presence of a pathogen in an environmental sample such as water. In an agricultural application, the predetermined molecular structure can provide an indication of crop resistance, for example. In a medical application, a predetermined molecular structure can be indicative of a disease gene.

Of particular interest is the detection of a predetermined nucleotide base sequence in a sample from a living organism or a plant. For a sample obtained from an individual, the sample can include a DNA sample or an RNA sample, for example. The predetermined nucleotide base sequence can be associated with a genetically-inherited disease and/or a genetic predisposition to disease of the individual, for example. Detection of these types of molecular structures allow medical personnel to formulate an appropriate treatment strategy for the individual.

As indicated by block 10, a step of providing a molecular detection device is performed. Various types of molecular detection devices can be utilized. Such molecular detection devices include, but are not limited to, molecular detection chips, DNA chips, biosensor arrays, genosensor arrays, and the like.

The molecular detection device typically includes a plurality binding sites or hybridization sites which are arranged as an array on a substrate. Each binding site has a respective molecular receptor which specifically binds or

hybridizes with a molecule having a predetermined structure.

Each molecular receptor typically includes a biological or synthetic molecule having a specific affinity to the molecule to be detected. Of particular interest is a molecular receptor having a chain of at least one nucleotide to hybridize with a molecule having a complementary chain of at least one nucleotide. Here, for example, the molecular receptor can include a DNA probe for detecting a corresponding, complementary DNA sequence in the sample.

It is noted, however, that the scope of the invention is not limited to sensing the hybridization of DNA molecules. For example, embodiments of the present invention can be utilized to detect RNA hybridization and antibody-antigen binding events. As another alternative, the molecular detection device can include an array of detection sites, such as in the context of an oligonucleotide ligation assay (OLA). Using a ligase chain reaction, pairs of oligonucleotides are utilized to amplify a selected oligonucleotide sequence. To detect the selected oligonucleotide sequence, a corresponding detection site is screened for full-length ligated oligonucleotides using any of the sensing approaches described herein.

As indicated by block 12, an optional step of tagging molecules within the sample is performed. Each molecule is tagged with a member which can be sensed by the molecular detection device. Such members are commonly referred to in the art as tags, markers, and labels. Examples of such members include, but are not limited to,

radioactive members, optical members (such as fluorescent members, luminescent members, and light-scattering members), charged members, and magnetic members.

As indicated by block 14, a step of applying the sample to the molecular detection device is performed. Thereafter, the sample is allowed to hybridize at one or more of the binding sites, as indicated by block 16. Typically, the sample specifically binds to at least one of the binding sites, and non-specifically binds to at least another one of the binding sites. By specific binding, it is meant that an intended target molecule is bound to a molecular receptor. By non-specific binding, it is meant that an unintended target molecule is bound to a molecular receptor. In the case where the molecular receptor includes a chain of at least one nucleotide, specific binding occurs with a molecule having a complementary chain of the at least one nucleotide. Non-specific binding with such a molecular receptor occurs with a molecule having at least one mismatching base.

To hasten the hybridization process, a local concentration of molecules in the sample can be increased at predetermined sites using electric field enhancements. Here, each site has an electrode associated therewith for selectively generating an electric field thereby. The electric field is generated by applying an electric potential between an electrode at the site and a counter electrode. To attract molecules to the site, the polarity of the electric potential is selected to generate an electric field having a polarity opposite to the charge of the molecules.

After hybridization, an optional step of removing unwanted molecules from the binding sites can be performed, as indicated by block 18. The step of removing unwanted molecules can be performed by generating an electric field having the same polarity as the charge of the unwanted molecules. The electric field acts to repel unwanted molecules from the binding sites. As an alternative to, or in conjunction with, the field-based approach, a thermally-assisted approach can be utilized to remove unwanted molecules. Here, the temperature at the binding sites is raised, in dependence upon a melting temperature, to dissociate partially-bound molecules from the molecular receptors. Regardless of the approach utilized, the unwanted molecules to be dehybridized can include unbound molecules and partially-bound (i.e. non-specifically bound) molecules.

Typically, the step of removing unwanted molecules does not remove all unwanted molecules from the binding sites. This step is beneficial, however, in improving the accuracy of detection as outlined in subsequent steps.

As indicated by block 20, the method includes a step of sensing a pattern in which the sample binds to an array of binding sites in a molecular detection device. The pattern can be sensed using a variety of approaches, including but not limited to, optical approaches, radioactive-sensing approaches, electronic approaches, and magnetic approaches. The specific approach utilized depends upon the type of tagging member attached to the molecules in the sample.

Preferably, the step of sensing the pattern includes sensing an intensity or a magnitude of

binding at each of a plurality of the array of binding sites. Each intensity can be indicative of a number of molecules bound at a respective binding site, a binding strength at a respective binding site, and/or a melting temperature for the binding at a respective binding site.

For the purpose of illustration, the method will be described using an optical sensing approach. Here, the step of sensing the pattern includes capturing at least one image of the pattern. Typically, the image is formed by luminescent light, fluorescent light or a scattering of light associated with a hybridization event. The image can be captured using a CCD camera, a confocal microscope, or other like imaging device.

Preferably, the image indicates an intensity or magnitude of binding at each of the binding sites by a measure of illumination. The measure of illumination at a binding site can be based on a gray scale or the like at the location of the binding site on the image. In one embodiment, the intensity of binding at a binding site is based on an average illumination (e.g. an average gray scale) at the binding site.

As indicated by block 22, the method includes a step of comparing the pattern to a reference pattern to detect the predetermined molecular structure in the sample. In one embodiment, the step of comparing includes determining a correlation between the pattern and the reference pattern. Here, the predetermined molecular structure can be detected when a measure of the correlation is within a predetermined range. In another embodiment, the step of comparing includes

determining a difference between the pattern and the reference pattern. Here, the predetermined molecular structure can be detected when a measure of the difference is within a predetermined range.

For optical sensing embodiments, the step of comparing includes a step of comparing at least one image of the pattern to an image of the reference pattern.

As indicated by block 24, the method optionally comprises a step of determining a confidence level of detecting the predetermined molecular structure in the sample. The confidence level indicates a degree of significance of the result obtained in the step of comparing in block 22.

To screen the sample for a plurality of different molecular structures, the steps indicated by blocks 22 and 24 can be repeated for a plurality of different reference patterns. Here, for example, a genomic sample can be screened to determine if it contains any of a plurality of predetermined base sequences.

FIG. 2 is a flow chart of an embodiment of a method of generating the reference pattern. Typically, the reference pattern is generated prior to performing the steps indicated in FIG. 1.

As indicated by block 30, the method includes a step of providing a reference device having a like array of binding sites as the molecular detection device used for detection. If desired, the same molecular detection device can be utilized for generating the reference pattern and for subsequent detection of an unknown molecular structure in a sample. Typically, however, another like device is utilized.

As indicated by block 32, an optional step of tagging molecules in a reference sample is performed. The reference sample is selected to contain the predetermined molecular structure which is to be detected.

As indicated by block 34, a step of applying the reference sample to the reference device is performed. As indicated by block 36, the reference sample is allowed to hybridize at one or more of the binding sites. Optionally, a step of removing unwanted molecules from the binding sites is performed, as indicated by block 38.

As indicated by block 40, a step of sensing a pattern in which the reference sample binds to the like array of binding sites is performed. This pattern is utilized as the reference pattern for subsequent comparison. Preferably, the reference pattern is sensed using the same approach as utilized for subsequent detection steps. It is further preferred that an intensity of binding at each of the binding sites in the like array be included in the reference pattern.

As indicated by block 42, an optional step of modifying a temperature at the binding sites is performed. Preferably, the temperature is raised to dissociate weakly bound molecules from the binding sites. Thereafter, the step of sensing the pattern, indicated by block 40, is repeated. The temperature can be repeatedly raised to generate a plurality of temperature-dependent reference patterns.

Thereafter, the reference device can be washed, and the steps indicated by blocks 32, 34, 36, 38, 40, and 42 can be repeated for another reference sample containing the same predetermined

molecular structure. Alternatively, the steps indicated by blocks 30, 32, 34, 36, 38, 40, and 42 can be repeated to apply the same predetermined molecular structure to a number of like reference devices. Either approach may be utilized to provide a plurality of reference patterns for the same predetermined molecular structure.

Sensed patterns formed by a sample having an unknown molecular structure can be compared to each of the above-described plurality of reference patterns, or a statistical model thereof, to detect the predetermined molecular structure in the sample.

FIG. 3 is a flow chart of another embodiment of a method of generating the reference pattern. As indicated by block 50, the method includes a step of determining an architecture of the array of binding sites of the molecular detection device. This step can include determining a layout of the binding sites, and determining the type of molecular receptor at each of the binding sites.

As indicated by block 52, the method includes a step of predicting a reference pattern in which the predetermined molecular structure binds to the array of binding sites. The reference pattern is predicted based upon the predetermined molecular structure and the architecture of the molecular detection device. Preferably, the reference pattern includes a predicted intensity of binding at each of the binding sites.

Regardless of the approach taken, the reference pattern acts as a novelty filter which is predictive of a successful or a desirable test result.

FIGS. 4 to 7 illustrate the detection of a predetermined molecular structure in accordance with a specific embodiment of the present invention. In this simplified example, a predetermined sequence of nucleotide bases is detected in a genomic sample, such as a DNA sample.

FIG. 4 illustrates an array of binding sites 60 on a molecular detection device used in the specific embodiment. The array of binding sites 60 contains all possible single-stranded DNA probes having a length of three bases. It is noted that molecular detection devices used in practice typically have more binding sites. For example, 256x256 arrays containing all possible 8-mer DNA probes are commonly utilized.

The respective base sequence at each binding site is indicated using standard nucleotide abbreviations ("a" representing adenine, "c" representing cytosine, "g" representing guanine, and "t" representing thymine). As is known in the art, each DNA probe is utilized to detect molecules having a complementary sequence of nucleotide bases.

FIG. 5 illustrates a reference pattern for detecting an a-c-g nucleotide sequence in a sample. The reference pattern indicates an intensity of binding at each of the binding sites by an intensity of illumination. Here, darker sites are indicative of lower binding intensities, while brighter sites are indicative of higher binding intensities.

A binding site 62 having a t-g-c sequence, which is complementary to the a-c-g sequence, has the greatest intensity of binding. Binding sites having single-mismatching complementary bases, such

as binding sites indicated by reference numeral 64, have a lesser intensity of binding. Those binding sites having two mismatching complementary bases, such as those indicated by reference numeral 66, have an even lesser intensity of binding. Binding sites with no matching complementary bases, such as those indicated by reference numeral 68, have a low intensity of binding.

FIG. 6 illustrates a reference pattern for detecting an a-c-t nucleotide sequence in a sample. The reference patterns in FIGS. 5 and 6 can be sensed using a reference sample, or can be predicted based on the number of mismatching bases at each binding site.

For purposes of illustration, the sequences in this example are assumed to have a specific orientation. As a result, an a-c-t sequence and a t-c-a sequence do not specifically hybridize at the same binding site. It is noted, however, that this should not be construed as a limitation in the scope of the present invention.

FIG. 7 is an example of a pattern generated by a sample having an unknown molecular structure. The pattern is generated by applying a sample of tagged single-stranded DNA molecules to the molecular detection device, allowing the molecules to hybridize to the binding sites, and optionally removing unwanted molecules.

The resulting pattern shows a high intensity of binding at a t-g-a site 70. If standard detection techniques were utilized, one would conclude that the sample includes an a-c-t nucleotide sequence (i.e. the complement of t-g-a). However, the overall pattern is better correlated to the reference pattern for the a-c-g nucleotide

sequence (in FIG. 5) than to the reference pattern for a-c-t (in FIG. 6). Moreover, the pattern is significantly correlated to the reference pattern in FIG. 5. Hence, the method of the present invention would conclude that the sample includes the a-c-g nucleotide sequence.

The pattern in FIG. 7 can be compared to the reference patterns in FIG. 5 and FIG. 6 using any of a variety of correlation measures and/or difference measures. For example, a difference measure can be formulated using intensity differences (between the pattern and the reference pattern) for each of the binding sites. The difference measure can be a sum of absolute differences, a maximum difference, a root-mean square difference, or any other suitable function of the intensity differences. A correlation measure can be formulated using a coefficient of correlation between the pattern intensities and the reference intensities. In general, any linear or nonlinear function of the pattern intensities and the reference intensities can be utilized to compare the pattern to the reference pattern.

FIG. 8 is a flow chart of additional steps which can be utilized to detect the predetermined molecular structure. The steps illustrated here can be performed after performing the steps indicated by blocks 10, 12, 14, 16 and 18 in FIG. 1.

As indicated by block 80, a step of modifying a temperature at the binding sites is performed. Preferably, the temperature is raised to dissociate weakly bound molecules from the binding sites. The temperature can be independently modified at

selected binding sites, or can be modified for all of the binding sites.

Thereafter, a step of sensing a pattern, indicated by block 82, is performed. By repeatedly raising the temperature and sensing a resulting pattern, a plurality of temperature-dependent patterns is generated.

As indicated by block 84, a step of comparing at least one of the plurality of temperature-dependent patterns to a corresponding at least one of a plurality of temperature-dependent reference patterns is performed. Here, for example, each of the temperature-dependent patterns can be compared to a corresponding one of the temperature-dependent reference patterns. Alternatively, only selected ones of the temperature-dependent patterns can be compared to corresponding reference patterns. A correlation measure and/or a difference measure is computed based on this comparison. A predetermined molecular structure is detected when the measure is within a predetermined range.

In one embodiment, the temperature-dependent pattern having a greatest variability of intensity is selected for comparison. The variability of intensity is greatest at a temperature which dissociates many non-specifically-bound molecules, but does not significantly dissociate specifically-bound molecules. This pattern can be compared with a corresponding reference pattern to detect a predetermined molecular structure. It is noted that a variety of different measures of variability can be utilized, including but not limited to, sample variance and sample standard deviation.

FIG. 9 is a block diagram of an apparatus for detecting a predetermined molecular structure in a

sample. The apparatus includes an array of binding sites 90 each having a respective molecular receptor. A sensor 92 provides means for sensing the pattern in which the sample binds to the array of binding sites 90. Various types of sensors can be utilized, as described earlier.

A memory 94 contains a library of at least one reference pattern. Preferably, the memory 94 contains a library of a plurality of reference patterns so that the apparatus can be utilized for detecting any of a plurality of predetermined molecular structures in the sample.

A processor 96 is operatively associated with the sensor 92 and the memory 94. The processor 96 provides means for comparing the pattern sensed by the sensor 92 to the reference patterns stored in the memory. Based on the comparison(s), the processor 96 outputs an indication of which predetermined molecular structure is detected, and a confidence level therefor.

In one embodiment, the array of binding sites 90, the sensor 92, the memory 94, and the processor 96 are integrated to form a single device. Alternatively, selected ones of the above-described components can be external to the device.

Regardless of its form, the above-described apparatus can be utilized to perform the steps described for FIGS. 1 and 8. To modify the temperature at the binding sites, one or more heating elements 98 can be incorporated in the device. The heating elements 98 are preferably controlled by the processor 96 to form a plurality of temperature-dependent patterns.

FIG. 10 is a flow chart of an embodiment of a method of gene discovery in accordance with the

present invention. As indicated by block 100, the method includes a step of sensing a pattern of detection for a sample applied to a molecular detection device having a plurality of detection sites. The sample is taken from a first species having unknown genes to be discovered. Any of the various approaches described herein can be utilized for sensing the pattern.

As indicated by block 12, the method includes a step of determining an architecture of the plurality of detection sites of the molecular detection device.

A step of reading a nucleotide sequence from a database is performed, as indicated by block 104. In general, any nucleotide sequence can be read. Of particular interest, however, is a nucleotide sequence from a second species other than the first species. For example, the nucleotide sequence can include a gene from a fruit fly, while the sample in which gene discovery is to be performed is from a human.

As indicated by block 106, the method includes a step of predicting a reference pattern which would be detected if the nucleotide sequence were applied to the molecular detection device. As indicated by block 108, a step of comparing the pattern to the reference pattern is performed to determine whether the nucleotide sequence is within the sample.

The steps of reading a nucleotide sequence from the database, predicting a reference pattern for the nucleotide sequence, and comparing the pattern to the reference pattern are repeated to discover the presence of genes across different species.

Thus, there has been described herein a concept, as well as several embodiments including preferred embodiments of a method and apparatus for detecting predetermined molecular structures in a sample.

Because the various embodiments of the present invention compare actual test patterns to predicted or sensed reference patterns, they provide a significant improvement in that the results of a diagnostic test can be rapidly determined. Additionally, the various embodiments of the present invention as herein-described use a plurality of temperature-dependent patterns to improve the sensitivity and accuracy of detection.

It will be apparent to those skilled in the art that the disclosed invention may be modified in numerous ways and may assume many embodiments other than the preferred form specifically set out and described above.

Accordingly, it is intended by the appended claims to cover all modifications of the invention which fall within the true spirit and scope of the invention.

Claims

1. A method of detecting a predetermined molecular structure in a sample, the method comprising the steps of:

sensing a pattern in which the sample binds to an array of binding sites in a molecular detection device; and

comparing the pattern to a reference pattern to detect the predetermined molecular structure in the sample.

2. The method of claim 1 further comprising the step of determining a confidence level of detecting the predetermined molecular structure in the sample.

3. The method of claim 1 wherein the step of comparing includes at least one of determining a difference between the pattern and the reference pattern, determining a correlation between the pattern and the reference pattern, comparing at least one image of the pattern to an image of the reference pattern, comparing a plurality of temperature-dependent patterns to a plurality of temperature-dependent reference patterns.

4. The method of claim 1 wherein an intensity level of at least one of the binding sites is indicative of at least one of molecules bound at a respective binding site, a binding strength at a respective binding site, and a melting temperature at a respective binding site.

5. The method of claim 1 wherein the sample includes at least one of an environmental sample and an agriculture sample.

6. An apparatus for detecting a predetermined molecular structure in a sample, the apparatus comprising:

a sensor for sensing a pattern in which the sample binds to an array of binding sites;

a memory which contains a reference pattern for the predetermined molecular structure; and

a processor operatively associated with the sensor and the memory, the processor for comparing the pattern to the reference pattern to detect the predetermined molecular structure in the sample.

7. The apparatus of claim 6 wherein the sensor senses the pattern by capturing at least one image of the pattern.

8. The apparatus of claim 6 wherein the sensor senses a plurality of temperature-dependent patterns.

9. The apparatus of claim 8 wherein the processor selects a temperature-dependent pattern having a greatest variability of intensity, and compares the temperature-dependent pattern having the greatest variability of intensity to the reference pattern.

10. The apparatus of claim 6 wherein the reference pattern is generated by sensing a pattern in which a reference sample containing the predetermined molecular structure binds to a like

array of binding sites or by predicting a pattern in which the predetermined molecular structure binds to the array of binding sites.

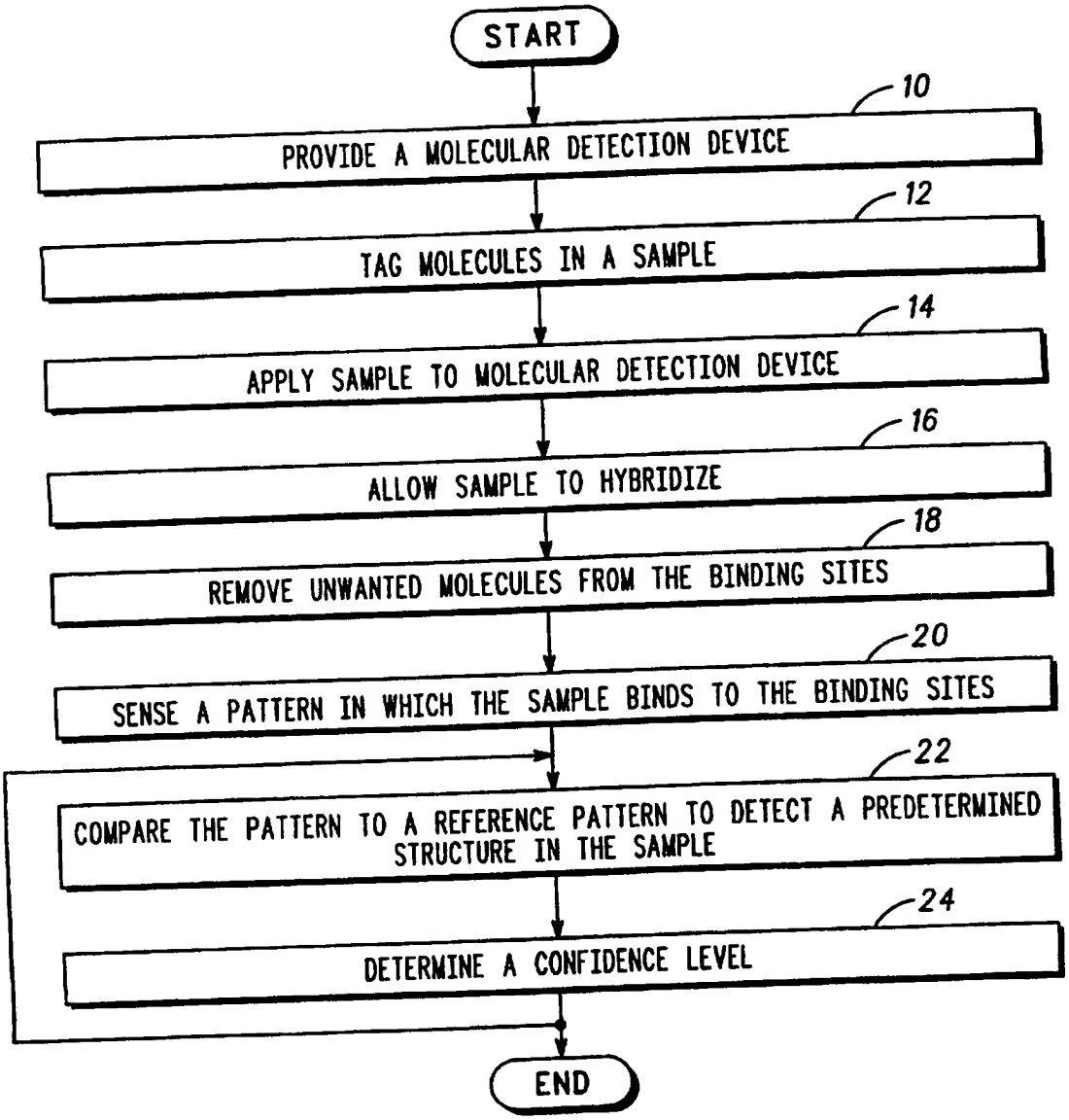
START
10
PROVIDE A MOLECULAR DETECTION DEVICE
12
TAG MOLECULES IN A SAMPLE
14
APPLY SAMPLE TO MOLECULAR DETECTION DEVICE
16
ALLOW SAMPLE TO HYBRIDIZE
18
REMOVE UNWANTED MOLECULES FROM THE BINDING SITES
20
SENSE A PATTERN IN WHICH THE SAMPLE BINDS TO THE BINDING SITES
22
COMPARE THE PATTERN TO A REFERENCE PATTERN TO DETECT A PREDETERMINED STRUCTURE IN THE SAMPLE
24
DETERMINE A CONFIDENCE LEVEL
END

FIG.1

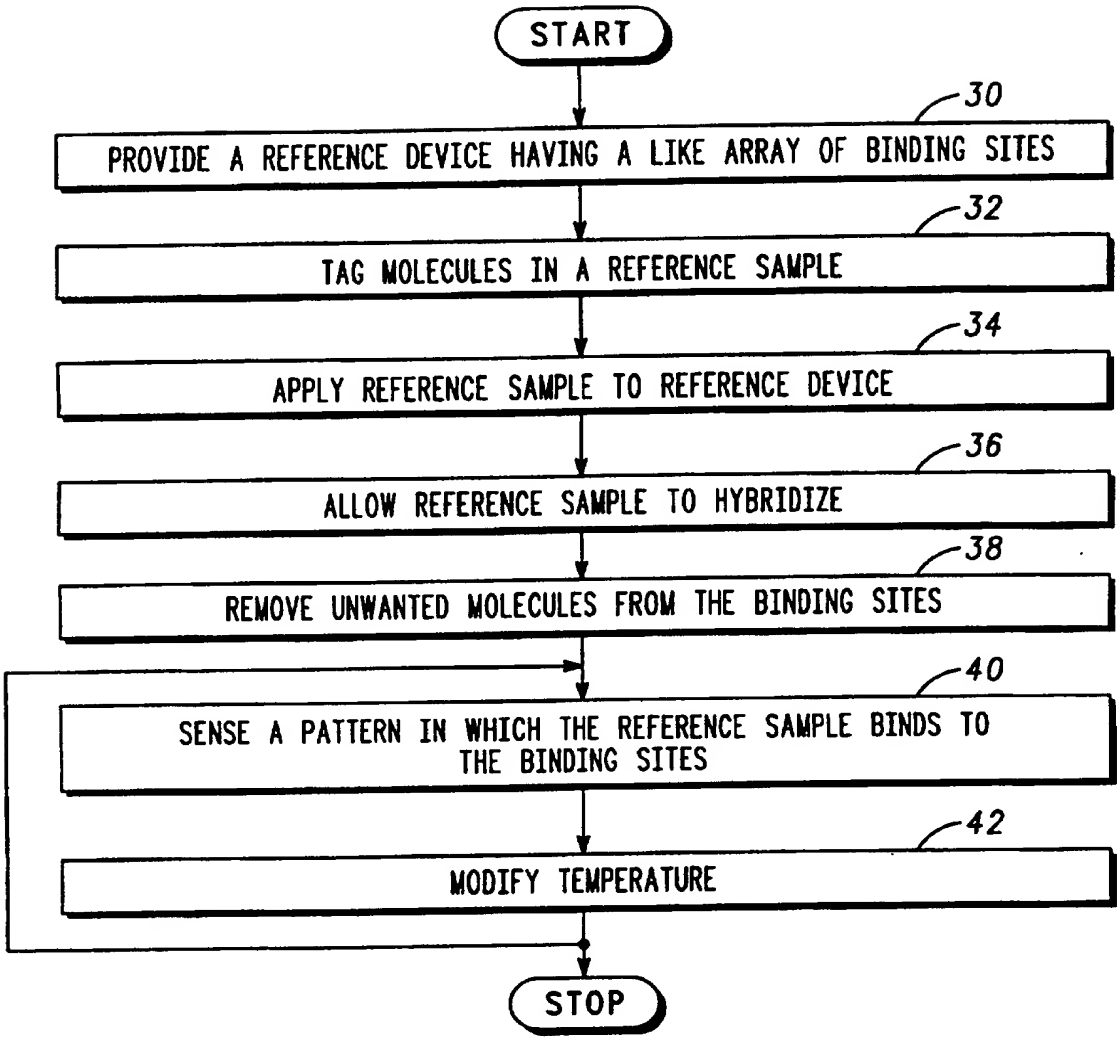
START
30
PROVIDE A REFERENCE DEVICE HAVING A LIKE ARRAY OF BINDING SITES
32
TAG MOLECULES IN A REFERENCE SAMPLE
34
APPLY REFERENCE SAMPLE TO REFERENCE DEVICE
36
ALLOW REFERENCE SAMPLE TO HYBRIDIZE
38
REMOVE UNWANTED MOLECULES FROM THE BINDING SITES
40
SENSE A PATTERN IN WHICH THE REFERENCE SAMPLE BINDS TO THE BINDING SITES
42
MODIFY TEMPERATURE
STOP

FIG.2

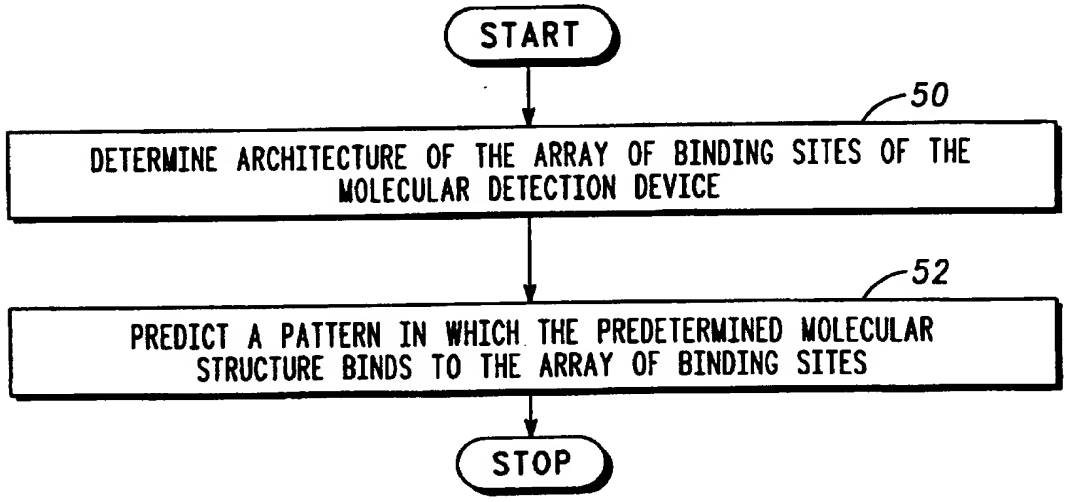
START
50
DETERMINE ARCHITECTURE OF THE ARRAY OF BINDING SITES OF THE MOLECULAR DETECTION DEVICE
52
PREDICT A PATTERN IN WHICH THE PREDETERMINED MOLECULAR STRUCTURE BINDS TO THE ARRAY OF BINDING SITES
STOP

FIG.3

AAA	AAC	ACA	ACA	CAA	CAC	CCA	CCC
AAG	AAT	ACG	ACT	CAG	CAT	CCG	CCT
AGA	AGC	ATA	ATC	CGA	CGC	CTA	CTC
AGG	AGT	ATG	ATT	CGG	CGT	CTG	CTT
GAA	GAC	GCA	GCC	TAA	TAC	TCA	TCC
GAG	GAT	GCG	GCT	TAG	TAT	TCG	TCT
GGA	GGC	GTA	GTC	TGA	TGC	TTA	TTC
GGG	GGT	GTG	GTT	TGG	TGT	TTG	TTT

60

FIG.4

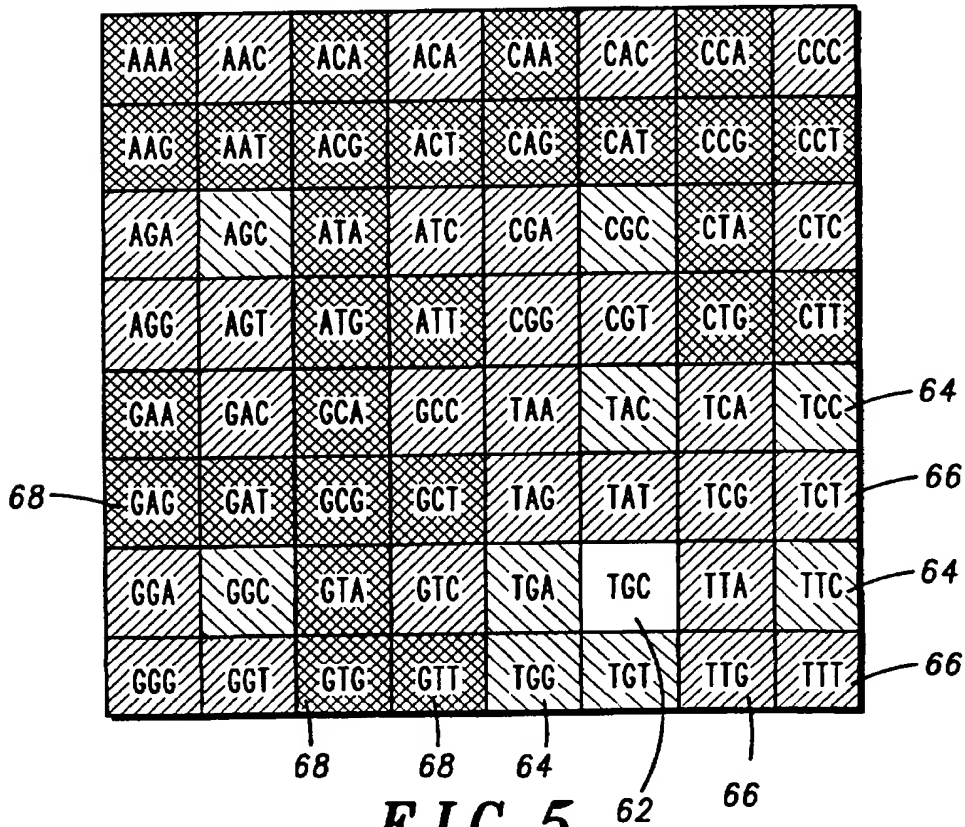

FIG.5

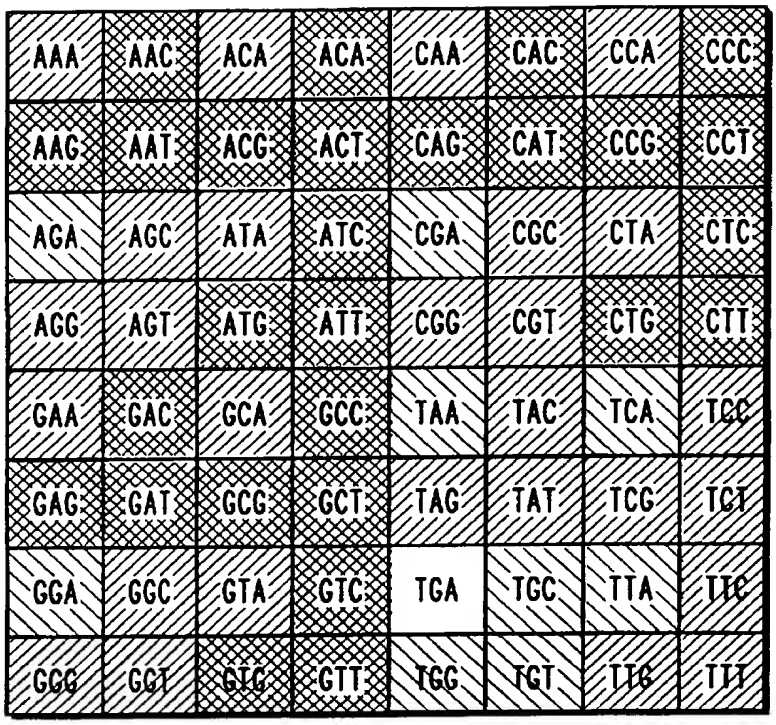
AAA AAC ACA ACA CAA CAC CCA CCC
AAG AAT ACG ACT CAG CAT CCG CCT
AGA AGC ATA ATC CGA CGC CTA CTC
AGG AGT ATG ATT CGG CGT CTG CTT
GAA GAC GCA GCC TAA TAC TCA TCC
GAG GAT GCG GCT TAG TAT TCG TCT
GGA GGC GTA GTC TGA TGC TTA TTC
GGG GGT GTG GTT TGG TGT TTG TTT

FIG.6

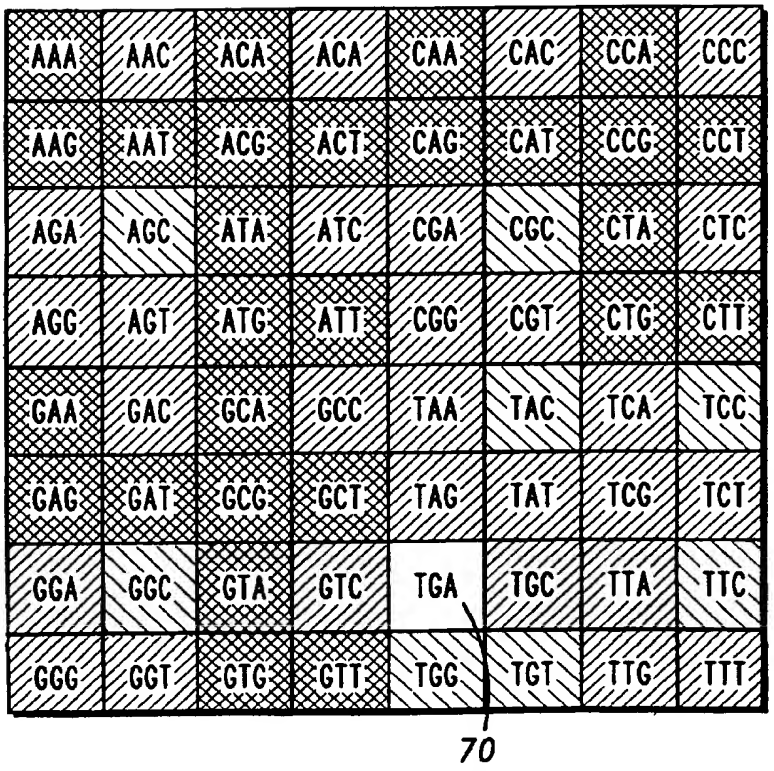
AAA AAC ACA ACA CAA CAC CCA CCC
AAG AAT ACG ACT CAG CAT CCG CCT
AGA AGC ATA ATC CGA CGC CTA CTC
AGG AGT ATG ATT CGG CGT CTG CTT
GAA GAC GCA GCC TAA TAC TCA TCC
GAG GAT GCG GCT TAG TAT TCG TCT
GGA GGC GTA GTC TGA TGC TTA TTC
GGG GGT GTG GTT TGG TGT TTG TTT
70

FIG.7

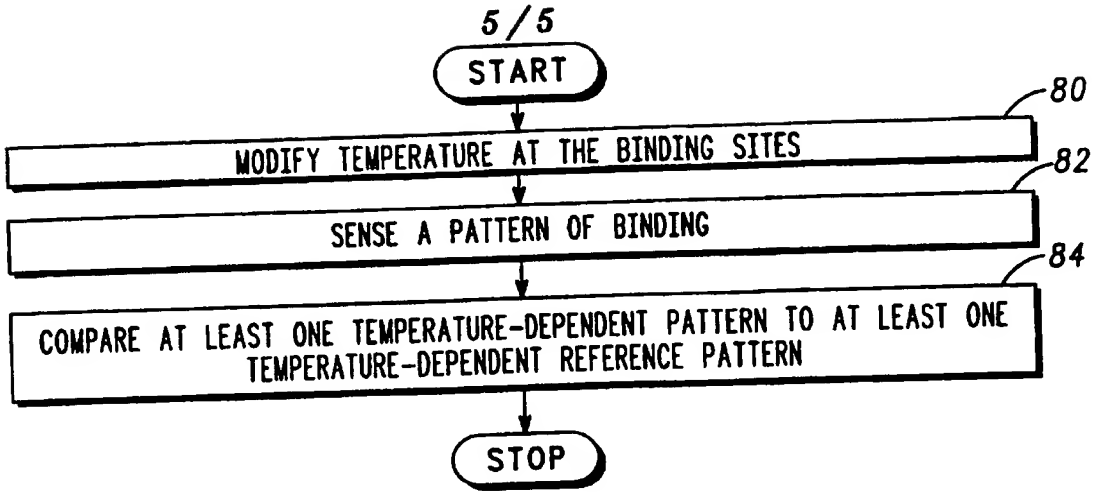

FIG.8

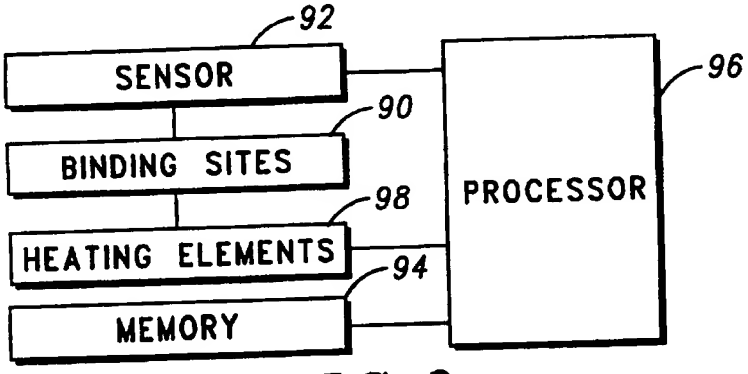

FIG.9

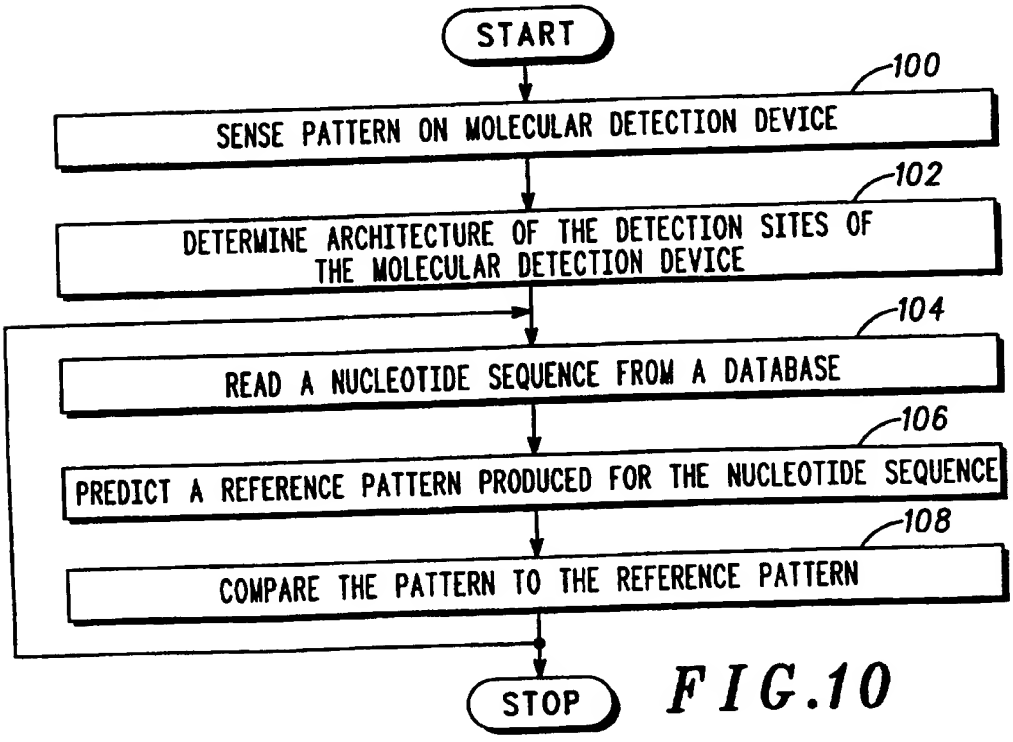

FIG.10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14372

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 204/153.1, 400, 403; 435/6, 283.1, 287.1, 287.2; 436/518, 524, 807, 809

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAPLUS, MEDLINE, WPIDS, SCISEARCH, MEDLINE, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,532,128 A (EGGERS et al) 02 July 1996. See entire document.	1-10
X	PEASE AC. Light-generated Oligonucleotide Arrays for Rapid DNA Sequence Analysis. Proc. Natl. Acad. Sci. May 1994. Vol 91. pages 5022-5026, especially Abstract.	1-10
X	STIMPSON DI. Real-time Detection of DNA Hybridization and Melting On Oligonucleotide Arrays by using Optical Wave Guides. Proc. Natl. Acad. Sci. July 1995. Vol 92. pages 6379-6383, especially Abstract	1-10
A,P	US 5,605,662 A (HELLER et al) 25 February 1997.	1-10

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
24 NOVEMBER 1997

Date of mailing of the international search report
23 DEC 1997

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14372

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	US 5,571,639 A (HUBBELL et al) 05 November 1996.	1-10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14372

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

G01N 27/00, 27/26, 33/543, 33/551; C12Q 1/68; A01N 1/02; C12M 1/00;

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

204/153.1, 400, 403; 435/6, 283.1, 287.1, 287.2; 436/518, 524, 807, 809